Appl. No. 09/975,139
Amdt. Dated April 26, 2006
Reply to Final Office action of October 28, 2005

Page 2

IN THE CLAIMS:

The claims as currently presented and under consideration, are presented below for the Examiner's convenience and to comply with 37 CFR §1.121. This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A method of creating a library of DNA sequences, said method comprising:

    a) providing a DNA sequence that encodes a protein of interest;

    b) providing a probability matrix for the protein;

    c) providing a constraint vector for the protein;

    d) applying the constraint vector to the probability matrix to produce a substitution scheme recommending substitutions of at least two residues in the protein; and

    e) creating a library of at least 25 DNA sequences incorporating changes in the DNA sequence that produce the recommended substitutions, wherein the library is produced in a bacterial host.

2. (Original) The method of claim 1, wherein said protein is selected from the group consisting of an esterase, dehydrogenase and hydrolase.

3. (Original) The method of claim 2, wherein said protein is selected from the group consisting of a protease, cellulase, lipase, hemicellulase, laccase, and amylase.

4. (Original) The method of claim 1, wherein said protein is selected from the group consisting of a transcription factor, growth factor, antibody, interleukin, antigen, and receptor.

5. (Original) The method of claim 1, wherein the probability matrix is based on structural characteristics selected from the group consisting of conservative residues, sequence alignments, three dimensional structure, residue environment, solvent accessibility, residue chemistry, propensity for a particular secondary structure, and combinations thereof.

GC637-2 Resp to FOA 4-26-06   2

PAGE 5/21 * RCVD AT 4/26/2006 3:00:56 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/12 * DNIS:2738300 * CSID:650 845 6504 * DURATION (mm-ss):04-42

Appl. No. 09/975,139
Amdt. Dated April 26, 2006
Reply to Final Office action of October 28, 2005

Page 3

6.   (Original) The method of claim 1, wherein the constraint vector is based on structural characteristics known to affect protein function selected from the group consisting of proximity to the site of functionality, distance of α or β carbons, contact with residues of interest, and contact with residues that contact the residue of interest.

7.   (Cancelled)

8.   (Cancelled)

9.   (Cancelled)

10.   (Cancelled)

11.   (Previously Presented) A method for creating at least one library of nucleic acid sequences that encode variants of a protein, said method comprising the steps of:

a)   identifying an initial nucleic acid sequence that encodes a desired protein;

b)   establishing a probability matrix for said protein, wherein said probability matrix is generated by a computer; and

c)   generating a constraint vector for said protein, wherein said constraint vector is generated by a computer; and

d)   applying said constraint vector to said probability matrix to produce a substitution scheme for use in step (e); and

e)   creating a library of at least 25 DNA sequences, wherein the library is created in a bacterial host.

12.   (Cancelled)

13.   (Cancelled)

14.   (Cancelled)

15.   (Previously Presented) The method of claim 1, wherein a library comprising at least 100 DNA sequences is produced.

Appl. No. 09/975,139
Amdt. Dated April 26, 2006
Reply to Final Office action of October 28, 2005

Page 4

16.   (Previously Presented) The method of claim 15, wherein a library comprising at least 250 DNA sequences is produced.

17.   (Previously Presented) The method of claim 16, wherein a library comprising at least 1000 DNA sequences is produced.

18.   (Previously Presented) The method of claim 17, wherein a library comprising at least 2500 DNA sequences is produced.

19.   (Previously Presented) The method of claim 18, wherein a library comprising at least 10,000 DNA sequences is produced.

20.   (Previously Presented) The method of claim 1, wherein a library of less than 10^9 DNA sequences is produced.

21.   (Previously Presented) The method of claim 20, wherein a library of less than 10^6 DNA sequences is produced.

22.   (Previously Presented) The method of claim 21, wherein a library of less than 10^5 DNA sequences is produced

23.   (Previously Presented) The method of claim 1, wherein the probability matrix is generated using an algorithm.

24.   (Cancelled)

25.   (Previously Presented) The method of claim 1, wherein the constraint vector is generated using an algorithm.

26.   (Original) The method of claim 1, wherein the constraint vector is generated by a computer.

Appl. No. 09/975,139
Amdt. Dated April 26, 2006
Reply to Final Office action of October 28, 2005

Page 5

27.   (Original) The method of claim 1, wherein the constraint vector is applied to the probability matrix using a computer.

28.   (Original) The method of claim 1, wherein the probability matrix is normalized.

29.   (Previously Presented) The method of claim 1, wherein the DNA sequence for either step (a) or step (e) is generated from DNA shuffling.

30.   (Cancelled)

31.   (Previously Presented) A method of creating a library of DNA sequences, said method comprising:

a)   providing a substitution scheme produced by applying a constraint vector to a probability matrix wherein the substitution scheme recommends substitutions at least two residues in a protein of interest; and

b)   creating a library of at least 25 DNA sequences incorporating substitutions in a DNA sequence encoding the protein of interest to create a library comprising the recommended substitutions, wherein said library is created in a bacterial host.